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Lung Lavage Neutrophils, Neutrophil Elastase and Albumin in the Prognosis of Pulmonary Sarcoidosis

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ABSTRACT

The authors measured the bronchoalveolar lavage fluid (BALF) neutrophils, neutrophil elastase and albumin in 60 patients with pulmonary sarcoidosis, 18 patients with other interstitial lung diseases and 14 healthy controls in order to evaluate its significance in the prognosis of sarcoidosis. The concentrations were measured by enzymoimmunoassay and radial immunodiffusion (LC plates). Obtained values were correlated with some clinical indices, radiological stage and lung function values. The patients were followed up for 22.3 6.8 months. At the end of observation period the outcome of sarcoidosis has been evaluated upon the findings of chest radiograms and lung function tests. The length of duration of the disease correlated with the lung lavage neutrophil counts ($r = 0.2535$; $p < 0.05$). Significantly higher values of BALF elastase were obtained in III radiologic stage than II and I. Patients with high BALF albumin had more often dispnoa ($p < 0.05$) and chronic forms of the disease ($p < 0.05$). The values were high in patients in II and III radiologic stage. Unfavorable outcome of chest radiograms was announced by high BALF albumins ($p < 0.05$). The measurement of BALF neutrophils, neutrophil elastase and albumin are helpful in clinical management and estimation of the prognosis in patients with pulmonary sarcoidosis.

Introduction

Sarcoidosis is a multiorganic granulomatous disease, which can invade almost any organ or tissue, but in 95% of the cases causes lung disease. It is a disease more common in nonsmokers[1], although most of other pulmonary[2] and nonpulmonary diseases[3] are more common in smokers. The prognosis of pul-monary sarcoidosis is good but approximately 10–15% of the cases progress to chronicity. Different clinical pictures have very variable prognosis, so for instance the acute cutaneous form erythema nodosum has 84% remission in two years

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time in contrast to chronic form of lupus pernio that has only 15%[4]. The prognosis of pulmonary sarcoidosis also depends on initial radiologic stage, hilar (I stage), hilopulmonary (II stage) or pulmonary (III stage), the prognosis of III stage being the worst, with overall 2 years remission of about 50%[5]. Lung function tests show restrictive, sometimes obstructive ventilatory changes[6], and diminished values of diffusion capacity, the latter being the omnious prognostic sign[7].

The analyses of various components of bronchoalveolar lavage fluid (BALF) have been a new tool to estimate the prognosis of pulmonary sarcoidosis by measuring at site events. The permanent inflammation and fibrosis of the pulmonary interstitium and peripheral airspaces are the hallmark of chronic events in sarcoidosis. This leads to derangement of the alveolar wall and loss of functional alveolar capillary units, resulting in a restrictive pattern in lung function tests. The development of fibrosis rests upon the activity of various inflammatory cells, lymphocytes, neutrophils, eosinophils and mast cells as well as pulmonary vascular leakage of proteins that may activate lung fibroblasts and initiate deposition of collagen in the lung interstitium[8]. The cell components have been vastly investigated. The lymphocytic alveolitis in sarcoidosis is a good prognostic sign, but the neutrophilic is not and may indicate chronicity[9]. Neutrophil elastases are the products of neutrophils and thus elevated might also be an omnious sign[10]. The raised albumin in lung lavage fluid is not the artifact due to BAL procedure as it was thought earlier but this finding is more likely to be related to the effects of chronic inflammatory changes in the lung due to underlying disease that causes increased lung vascular permeability[11]. The increased BALF albumin values have been found in interstitial lung diseases[12] and sarcoidosis[13], and these finding is in accordance with on going inflammatory changes. The prognosis of sarcoidosis is unpredictable in numerous cases. Our goal was to investigate whether the measurement of lung lavage neutrophils, neutrophil elastase and albumin can predict the outcome of pulmonary sarcoidosis.

Materials and Methods

Sixty patients with biopsy proven pulmonary sarcoidosis were included in the study. Al the patients were admitted at University Hospital for Lung Diseases »Jordanovac« in Zagreb. We performed thorough clinical examination with special concern for the duration of the disease prior to admittance, symptoms (cough, dispnoa), and way of presentation, acute or initially chronic. In all the patients chest radiograms and lung function tests (spirometry, body pletysmography and diffusion capacity) were performed. Bronchoalveolar lavage was done as a routine procedure at fiberoptic bronchoscopy. The differential cell count and biochemical analyses, neutrophil elastase and albumin of supernatant of lavage fluid were measured. The patients were followed up for 22.3 6.8 months (median 24). At the end of observation period the outcome of sarcoidosis has been evaluated upon the findings of chest radiograms and lung function tests. Chest radiograms and lung function tests (VC-vital capacity, TLC-total lung capacity, RV-residual volume and DLCO-diffusion capacity) were evaluated as normal or pathological at the end of observation period.

Bronchoalveolar lavage was performed in accordance with technical recommendations and guidelines of European Task Group[14]. The procedure was completed in a segment of the right middle lobe with 200 ml isotonic saline. The returned fluid (BALF) was filtered through two layers of cotton gauze. Differential cell counts were assessed by numbering

200 cells, and the results were expressed as percentage. The biochemical parameters were studied in a supernatant that remained after the centrifugation of lavage fluid. The concentration of neutrophil elastase in a complex with -1-protease inhibitor (-Pi) was measured by commercial enzymoimmunoassay (E. Merck, Darmstadt). BALF albumin concentrations were measured by radial immunodiffusion on low concentration plates (LC) (Behringwerke, Marburg).

The values were expressed as mean standard deviation, rarely as median values. For statistical analyses we used Chi-square test, Students t test, Wilcox-Mann-Whitney and Kruskal-Wallis test. The classical correlation tests and Spearmans rang correlations were also performed[15].

Results

The experimental group consisted of 60 patients with biopsy proven pulmonary sarcoidosis (S) and two control groups of 18 patients with other interstitial lung diseases (I) and 14 healthy persons (HP). Twenty two sarcoidosis patients were male and 38 female, with average age of 41.9 12.2 years. Table 1 shows the characteristics of the groups; there was not statistically significant difference between the groups concerning the age, gender or the smoking habits. Twenty three (38.3%) patients with pulmonary sarcoidosis were at I radiologic stage, 30 (50%) at II and 7 (11.7%) at III radiologic stage.

All the patients underwent bronchoalveolar lavage. Table 2 shows the results. The volume of recovered lavage fluid was equal in all three groups (S 104 21.4 ml, I 100.5 18.9 ml and HC 100.7 29.9 ml) as was the percentage of neutrophils (S 1.9 3.9 %, I 2.2 4 % and HC 0.6 0.9 %). The concentrations of neutrophil elastase (S 53.3 56.6 ng/nL, I 41.4 38.2 ng/mL, HC 20.5 10.2 ng/mL) ($p < 0.05$) and albumin (S 74.5 31.6 g/mL, I 66.5 31.6 g/mL and HC 28.8 8 g/mL) ($p < 0.001$) were statistically

TABLE 1
CHARACTERISTICS OF THE EXPERIMENTAL AND CONTROL GROUPS

Groups		Age (yrs)	Sex		Smoking	
			M	F	M	F
Sarcoidosis	N=60	41.9±12.2	22	38	44	16
Interstit.	N=18	48.3±13.4	11	7	11	7
Controls	N=14	43.9±9.3	7	7	11	3
Difference between groups		n.s.	n.s.		n.s.	

TABLE 2
NEUTROPHILS, NEUTROPHIL ELASTASES AND ALBUMIN IN BAL

	Sarciodosis	Interstit.	Controls	
VOF (mL)	104±21.4	100.5±18.9	100.7±29.9	n.s.
N (%)	1.9±3.9	2.2±4.0	0.6±0.9	n.s.
NE (ng/mL)	53.3±56.6	41.4±38.2	20.5±10.2	p<0.05
A (μg/mL)	74.5±31.6	66.5±31.6	29.8±8.0	p<0.001

VOF – volume of recovered lung fluid; N – neutrophils; NE – neutrophil elastases; A – albumin.

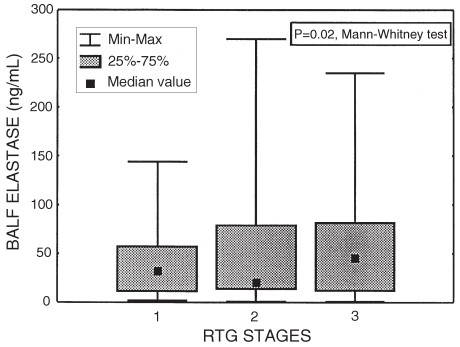

Fig. 1. BALF elastases according to the radiologic stages.

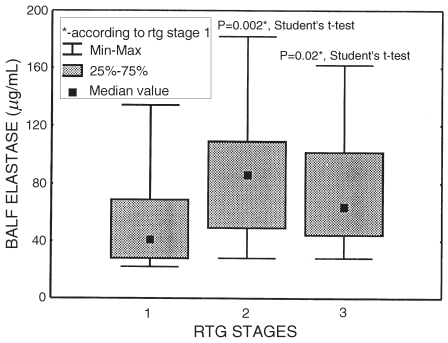

Fig. 2. BALF albumin according to the radiologic stages.

TABLE 3
CORRELATION OF CLINICAL DATA WITH INFLAMMATORY BALF INDICES

	Ly > 15%	N > 2%	ELA > 41 ng/mL	ALB > 46 μg/mL
Duration of the disease > 2 years	< 0.05	ns	ns	ns
Cough	< 0.05	ns	ns	ns
Dispnoa	ns	ns	ns	< 0.05
Smoking	ns	ns	ns	ns
Chronic presentation	ns	ns	ns	< 0.05
Corticosteroid therapy	ns	< 0.05	ns	< 0.01

Ly – lymphocytes; N – neutrophils, ELA – neutrophil elastases; ALB – albumin.

significantly higher in patients with sarcoidosis than those of the healthy controls.

We correlated some of the clinical indices with the results of BALF analyses (Table 3). The disease that persisted more than 2 years at the initial examination, cough and smoking was not correlated with BALF neutrophils, neutrophil elastase or albumin concentrations. Dispnoa and chronic initial presentation were correlated with high albumin concentrations ($p < 0.05$), and necessity for corticosteroid therapy with high BALF neutrophils ($p < 0.05$) and albumin ($p < 0.01$). The BALF experimental indices were observed in patients in different radiologic stage, I, II and III. The BALF elastase showed statistically significant higher values in the III radiologic stage than in the I (Figure 1), and BALF albumin concentrations were also higher but in the II ($p < 0.01$) and the III ($p < 0.05$) radiologic stage (Figure 2).

The patients were followed up for 22.3 6.8 months (median 24). Only the BALF neutrophils showed correlation with the duration of the disease ($p < 0.05$) (Figure 3). Control chest radiograms and lung function tests assessed the outcome. Only the high initial values of BALF albumin predicted the poor outcome of chest radiograms in pulmonary sarcoidosis ($p < 0.01$) (Figure 4).

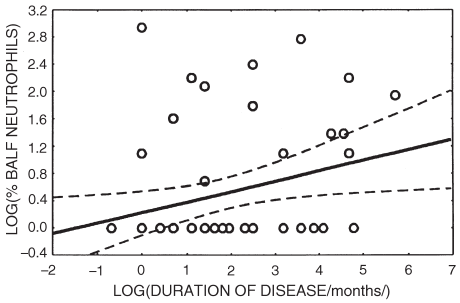

Fig. 3. Correlation of BALF neutrophils and duration of the disease.

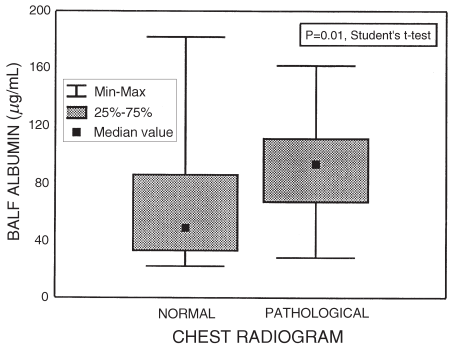

Fig. 4. Outcome of a chest radiogram according to the level of BALF albumines.

Discussion

Sarcoidosis is a very interesting disease for investigators because its ethiology, pathogenesis and prognosis are still unknown. The course of the disease in most cases is benign and has good prognosis. The drugs of choice are corticosteroids[16], but they have many unwanted, sometimes very serious side effects. It is very difficult in some cases to indicate such therapy especially when it is known that many cases will heal spontaneously. One and exact predictive finding in sarcoidosis does not exist.

The technique of lung lavage has enabled us to measure the at site events. The increased number of BALF neutrophils has been suggested to be the sign of chronicity. We did not find elevated values in chronic sarcoidosis as some other already mentioned authors have, but in the follow up period of approximately two years we found that the number of BALF neutrophils increased in time. Drent et al.[9] found that the number of neutrophils in bronchoalveolar lavage fluid distinguish between sarcoidosis patients who demonstrated remission and those having a more sever course. Patients with higher percentage of BALF neutrophils in our study were more often treated with corticosteroids and that also indicates advanced disease.

The exact role of proteolitic enzymes in BALF of sarcoidosis patients is not understood thoroughly, neither that of elastase nor the collagenase. The neutrophil elastases are increased in BALF according to some authors and our findings. Elastase from neutrophils has been linked to the pathologic processes of a variety of inflammatory diseases, for instance idiopathic pulmonary fibrosis. The serine proteinase has broad substrate specificity and may attack a number of host proteins outside of the neutrophil, including lung elastin and fibronectin. Such a proteolysis may change the normal surrounding tissue and protein pattern of an inflammatory focus[17]. The direct measurements of elastase in granulomatous tissue show that the enzyme activity increases along with granuloma maturation and that they cause the disappearance of elastic fibres and other elements of matrix[18]. Extrapolating this data it would be expected that elevated elastase means tissue damage, but we did not prove that increased BALF values announce progressive fibrotic disease. We showed only that they are correlated with radiologis stage; the values are higher in the III stage then in II and I. Such results correspond with findings of other authors[10].

The BALF albumin is increased in sarcoidosis. Clinical signs of chronicity correlated with high values, it also showed higher values in more advanced radiologic stages and finely it announced pathologic chest radiogram at the end of the study. All these findings show that the increased values are the sign of chronicity and that they announce progressive, fibrotic disease. We did not find any study in the literature that evaluated the correlation of clinical data and high BALF albumin values in pulmonary sarcoidosis.

This study was designed to evaluate the measurements of parameters at the site of the events, i.e. inside the lungs of sarcoidosis patients in order to estimate its prognosis by means of bronchoalveolar lavage. The increased values of BALF neutrophils correlated with the duration of the disease and thus showing its part in chronic progression. The BALF elastase were higher in more advanced radiologic stage, but did not show correlation with the indices of chronicity. The BALF albumin measurements were the most helpful in this respect because they announced the progressive disease and pathologic radiograms at the end of observation. We can conclude that these measurements are helpful in clinical judgment and estimation of prognosis in patients with pulmonary sarcoidosis.

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NEUTROFILNI GRANULOCITI, NEUTROFILNA ELASTAZA I ALBUMIN U PROGNOZI PLUĆNE SARKOIDOZE

S A Ž E T A K

Autori su određivali neutrofilne granulocite, neutrofilne elastaze i albumin u bronhoalveolarnom lavatu 60 bolesnika s plućnom sarkoidozom, 18 bolesnika s drugim intersticijskim bolestima pluća i 14 zdravih ispitanika sa ciljem da utvrde njihov značaj u procjeni prognoze sarkoidoze. Koncentracije su mjerene encimoimunoesejem i radijalnom imunodifuzijom. Dobivene vrijednosti korelirane su sa kliničkim pokazateljima, radiološkim stadijem i rezultatima testova plućne funkcije. Bolesnici su praćeni 22.3 6.8 mjeseci. Na kraju ispitivanja procijenjen je ishod sarkoidoze ma temelju nalaza radiograma toraksa i plućnih funkcija. Trajanje bolesti bilo je u pozitivnoj korelaciji s postotkom neutrofilnih granulocita u lavatu ($r = 0.2535$; $p < 0.05$). Signifikantno više vrijednosti elastaza u lavatu dobivene su u III nego u II i I radiološkom stadiju. Bolesnici s povišenim albuminima češće su imali dispneju ($p < 0.05$) i inicijalno kroničnu formu bolesti ($p < 0.05$). Više su vrijednosti dobivene u II i III radiološkom stadiju nego u I. Povišene vrijednosti albumina u lavatu označavale su patološki radiogram pluća nakon dvogodišnjeg praćenja. Mjerenje neutrofilnih granulocita, elastaza i albumina u bronhoalveolarnom lavatu je korisno u kliničkom postupku i procjeni prognoze bolesnika s plućnom sarkoidozom.